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Toxicology of Carbon Nanotubes - A Review

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Abstract

A large number of nanoparticles are present in the environment in which some are unintentionally produced; ultra fine particles or intentionally produced engineered nanoparticle (ENPs). The carbon based ENPs include single-walled and multi walled carbon nanotubes (SWCNTs and MWCNTs), spherical fullerenes and dendrimers. Among all ENPs, the carbon based ENPs are attracting much attention for potential biomedical applications, such as biosensors design, drug design, drug delivery, tumor therapy and tissue engineering, because of their electronic, optical and mechanical properties. The pristine CNTs are inert in nature so it needs to be functionalized to make it reactive. The functionalization appends different functional group e.g. C=O, C–O, –OH and –COOH to CNTs, which make it dispersible and suitable for different applications. The biocompatibility of these functionalized CNTs and their composite has to be tested before real time applications in the biological system. Determining the toxicity of CNTis the most persistent questions in nanotechnology. Inconsistent reports on toxicity of CNTs often appear in the literature and a mechanistic explanation of the reported toxicity remains incomprehensible.Results from various scientific tests on cells have so far proven confusing, with some results indicating it to be highly toxic and others showing no signs of toxicity. Several toxicity mechanisms have been proposed for CNTs including interruption of trans membrane electron transfer, disruption/penetration of the cell envelope, oxidation of cell components, and production of secondary products such as dissolved heavy metal ions or reactive oxygen species (ROS).Toxicity of a CNT sample is dependent on its composition along with its geometry and surface functionalization. Several studies have suggested that well-functionalized CNTs are safe to animal cells, while raw CNTs or CNTs without functionalization show severe toxicity to animal or human cells at even moderate dosage.

Keywords: *Carbon Nanotubes; In vitro toxicity; In vivo toxicity; Nanotechnology; Nanotoxicology.*

1. INTRODUCTION - CURRENT TOXICOLOGICAL KNOWLEDGE OF CARBON NANOTUBES

In the past several years, a significant number of studies have been made to study the toxic effects of carbon nanotubes. There are variations in the elucidations of these reports, it mainly depend on the type of nano materials as well as functionalization methods. Properly functionalized carbon nanotubes were shown non-toxic to animals conducted by various groups (Kam *et al.* 2004; Dumortier *et al.* 2006; Schipper *et al.* 2008; Wu *et al.* 2008), where as, raw carbon nanotubes were shown to be toxic to mice lungs in an*invivo* studies (Lam *et al.* 2004; Warheit *et al.* 2004; Shvedova *et al.* 2005 and Muller *et al.* 2005). Latest research revealed that non-functionalized, long MWNTs might be carcinogenic to mice (Poland *et al.* 2008). Pristine nanotubes are indicated to cause oxidative stress and decrease cell viability (Cui *et al.* 2005 ; Manna *et al.* 2005), however, there is some sign that leftover catalyst particles also contribute to this effect (Kagan *et al.* 2006). The cytotoxicity can be decreased to zero via functionalization with a covalently attached polar functional group (Sayes *et al.* 2006). Similarly, the toxicity of noncovalently functionalized carbon nanotubes depends on the nature of the functional group. Cells were viable upon

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internalization of individually encapsulated DNA-wrapped SWNTs complex (Kam *et al.* 2005).

Hence, carbon nanotube toxicity depends on the type of functionalization, aggregation behavior and the presence of metal catalyst particles leftovers during synthesis.

2. AN OVERVIEW OF CARBON NANOTUBES RESEARCH

Fabricating structural components with high strength to weight ratio using carbon nanotube polymer composite is the present focus of the researchers. One of the promising applications of polymer nanocomposite is the CNT reinforced ultra fine fiber via electrospinning (Rajendran *et al.* 2012; Formhals, 1934), which has been known since the 1930's.

Today, polymer fibers with nanometer diameter can be produced reasonably using the electrospinning technology with less than 100 nm diameters; these fibers are being studied for use in drug delivery systems, energy storage, and improved functional garments (Reneker *et al.* 1996; Kenawy *et al.* 2002; Laxminarayana *et al.* 2005). These applications require improved (i) fiber strength, (ii) thermal conductivity and (iii) electrical conductivity. Incorporating carbon nanotubes (CNTs) within electrospun fibers offers the probability of simultaneously improving all these three properties Ko *et al.* 2003; Dror *et al.* 2003; Lim *et al.* 2006). Xie *et al.* (2005) reviewed the dispersion and alignment of CNTs in polymer matrix.They found that the serious challenge is the development of means and ways to promote and increase the dispersion and alignment of CNTs in the matrix. Enhanced dispersion of CNTs in the polymer matrixwill foster and extend the applications and developments of polymer/CNT nanocomposites. Though optical detection techniques are possibly the most customary in biology and life sciences, electrochemical or electronic detection techniques have also been used in biosensors/biochips due to their great sensitivity, high specificity, and low cost. These techniques include voltammetric techniques (cyclic voltammetry and differential pulse voltametry), chronocoulometry, electrochemical impedance spectroscopy and electronic detection based on electric field (Cai *et al.* 2003). The sensors developed from CNTs have shown the ability to detect a range of analytes such as particular DNA sequences (Tu *et al.* 2009) cancer biomarkers (Thakare *et al.* 2010) and larger entities such as viruses (Patolsky *et al.* 2004). These sensor devices have also been used to monitor enzymatic activities and study the behavior of potential drug molecules (Prato *et al.* 2008). The detection of the analytes occurs with high specificity and sensitivity in rationally short time.Both SWCNT and MWCNT can be modified and conjugated to a bioactive molecule and biological species including carbohydrates, amino acids and peptides, nucleic acid, and proteins, for diverse biological applications. These biological applications are possible only because the carbon nanotubes possess some unique properties like one dimensional structure, high aspect ratio, excellent mechanical properties and chemical inertness (Endo *et al.* 2008). The carbohydrate functionalized carbon naotubes has already been used for recognition of pathogenic microorganisms namely, *E.coli* (Elkin *et al.* 2005) and *B. anthrasis* (Wang *et al.* 2008).

With respect to energy generation and storage, nanotubes show great potential in supercapacitors (Kim *et al.* 2006), Li-ion batteries (Laroux *et al.* 1999), solar cells (Landi *et al.* 2005) and fuel cells (Wang *et al.* 2004). Energy applications could become the largest applications domain in the bulk application of nanotubes. For the improvement of Li-ion batteries performance, MnO_2 and $LiFePO_4$ are being used as cathode while MWCNTs and graphene as anode. In the field of fuel cell, proton exchange membrane fuel cells (PEMFCs) have been extensively investigated (Kamavaram *et al.* 2009). In a PEMFC, the conversion of chemical energy to electrical energy occurs via a direct electrochemical reaction, and its efficiency is directly dependent on the catalysts used (Wang *et al.* 2005). The catalysts should have high stability, low cost and higher activities in oxygen reduction and/or fuel oxidation reaction (Kundu *et al.* 2005). Presently, the most commonly used catalysts in the PEMFCs are metal NPs, mainly Pt and/or Pt based alloys because of high oxygen reduction and/or fuel oxidation reaction due to high surface to volume ratio and better Fermi levels for redox reactions (Li *et al.* 2002). However, metal NPs are generally unstable and lost of their catalytic activity due to their irreversible aggregation during the electrochemical processes. Hence, exclusive supports are needed to mobilize and prevent these metal NPs from aggregation e.g., Carbon nanotubes (CNTs) are the most widely used support system in the current

scenario. Though the development of PEMFCs is under commercializationprocess, challenges including, how the CNTs affect the catalytic activity of the metal/CNTs and high materials cost persist. Development of more highly efficient catalysts with low cost for fuel cell commercialization would be one of the significant researches in this area. Because of the increased production and proposed use of CNTs in consumer products, there is a need for assessment of the potential toxicity of these nanoparticles.

3. *IN VIVO* TOXICITY STUDIES OF CARBON NANOTUBES

In vivo toxicity studies played asignificant role in risk assessment. These methods can be used to measure: acute toxicity, chronic toxicity, developmental toxicity, genotoxicity and reproductive toxicity. In *vivo* investigation is vital in the areas of medicine including cancer treatment. Many animal experiments are accomplished to highlight the potential harmful effects of newly developed medicines and chemical substances on human. In some cases, researchers try to mimic situations affecting human (e.g. arthritis, cystic fibrosis and cancer) in animals, to evaluate the abilities of new medicines in treating them.

To address the possible side effects of CNTs on human health and environment, animal models have been used to studied the toxicology of CNTs. Non-functionalized CNTs were introduced intratracheally (IT) into animals, manifestedas pulmonary toxicity including, inflammation and fibrotic reactions due to the accumulation of raw CNTs in the lung airways (Lam *et al.* 2004). Those results recommend that aerosol exposure of raw CNTs in the workplace should be avoided to protect human health. However, intratracheal instillation of functionalized soluble CNTs has little inference to the toxicology profile. In a latest pilot study, asbestos like pathogenicity was observed by Poland *et al.* (2008). when the mesothelial lining of the body cavity of mice was exposed to large MWCNTs of 80 - 160 nm diameter and 10 - 50 nm length (Poland *et al.* 2008). Yet the implication of this finding for possible negative effects of CNTs to human health is inadequate. It should be noted that the MWCNT materials used in this study were simply sonicated in bovine serum albumin (BSA) without surface functionalization. Moreover, no observable toxic effect was seen for shorter and smaller MWCNTs of 1-20 nm length and 10 - 14 nm diameter, acclaiming that the toxicology profiles of CNTs may differ between CNTs of different sizes. It is worth stating that functionalized SWCNTs used in biomedical research have length: 50 - 300 nm and diameter: 1 - 2 nm, which is entirely different from the geometry of MWCNTs used by Poland *et al.* (2008). Gambhir and group used covalently and noncovalently PEGylated SWCNTs to study the*in vivo* toxicity (Schipper *et al.* 2008). The PEGylated SWCNTs (~3 mg kg^{-1}) were intravenously injected into mice and observed over four months.Systolic blood pressure, total blood counts and serum chemistry recorded every month. Necropsy and tissue histology examinations were performed at the end of four months. The blood chemistry and histological observations were normal.These experiments suggest that functionalized biocompatible SWCNTs may be safe for *in vivo* biological applications. Another study showed similar results, supporting that PEGylated SWCNTs are slowly excreted from the body after systemic distribution in mouse models, without exhibiting obvious toxicity in the process (Liu *et al.* 2008). Yang *et al.* (2008) revealed that SWCNTs suspended in Tween-80, showed low toxicities to the tested mice at a high dose of ~40 mg kg^{-1}, following intra venous administration for three month. Toxicity may be due to the oxidative stress produced by SWCNTs accumulated in the liver and lungs of mice (Yang *et al.* 2008). The toxicity revealed was dose-dependent, and seemed to be less understandable at lower doses. A recent report by the same group revealed that covalently PEGylated SWCNTs, exhibited an ultralong blood circulation half-life in mice. Though the long-term toxicology of modified SWCNTs is yet to be examined. No acute toxicity has been reported even at a higher dose of 24 mg kg^{-1}.

3.1 Respiratory toxicity

Aguinea pigs was instilled intratracheally with the soot of CNT. Breathing frequency, tidal volume, pulmonary resistance, bronchoalveolar fluid and protein content were measured. The authors revealed that working with soot containing CNT was possibly not a health hazard, butthey did not perform his topathological study (Huczko *et al.* 2001). A study in mice is performed by Lam *et al.* (2004) and they established that SWCNT could be toxic if they reached the lungs; Warheit *et al.* (2004) conducted a similar study in rats, relating the

granuloma formation probably due to aggregation of CNT. Muller *et al*, compared carbon black, MWCNT and asbestos effects, instilled in the trachea of rats. Authors explained dose-dependent inflammation and granuloma formation, more substantial with MWCNT than with carbon black than asbestos. Exposer of SWCNT in mice resulted in early granulomatous reaction, uncommon acute inflammatory response and progressive fibrosis. Here pharyngeal aspiration was used instead of the intratracheal instillation used in the earlier studies, and allowed aerosolization of fine SWNCT particles. Another recent study suggests changes in deposition pattern and pulmonary response when SWCNT are evenly dispersed in the suspension prior to pharyngeal aspiration (Mercer *et al.* 2008). A new research suggest, MWCNTs migration to the subpleura and associated increased number of pleural mononuclear cells and subpleural fibrosis in mice upon inhalation (Ryman- Rasmussen *et al.* 2009), further caution and proper security measures are recommendedwhen handling CNT. Study by Wang *et al.* (2010), supports the previous reports, they described *in vitro* and *in vivo* stimulation of collagen deposition, lung fibroblasts proliferation, and metalloproteinase 9 increased expressionwithout inflammation when dispersed SWCNT (DSWCNT) was used Wang *et al.* (2010). Following inhalation, other type of nanoparticles may reach the central nervous system (CNS) Elder *et al.* (2010) by a process called transcytosis (Zensi *et al.* 2009). Studyrevealed that inhaled gold nanoparticles accumulate in olfactory bulb of rats and reach the cerebral cortex, lung and theother organs such as tongue, esophagus, kidney, spleen, aorta, septum, heart and blood (Yu *et al.* 2007). These observations advocate that nanoparticles can enter into the CNS via the olfactory nerve if there are present in high doses in the air. These nanoparticles may effects not only on respiratory tract and neighboring organs but spread to distant organs.

3.2 Bio-distributionof carbon nanotubes

Understanding bio-distribution of CNTs after systemic administration into animals is a very important consideration. Many scientists carried out *in vivo* bio-distribution and pharmacokinetic studies in the past few years. They used different CNT materials, surface functionalizations methods and tracking methodologies. Subsequentlythey obtainedvariable and sometimes questionable results. Singh *et al.* (2006) and Lacerda *et al.* (2008) used radiolabeled (^{111}In-DTPA) SWCNTs and MWCNTs to explain bio-distribution (Singh *et al.* 2006). Remarkably, after intravenous injection of CNTs into mice, no uptake in reticulo-endothelial system (RES) such as the liver and spleen was observed. However, fast urinal clearance of CNTs was observed. >95 % of CNTs were cleared within 3 hrs. These result sare similar to the *in vivo* behavior of small molecules, but different from that predicted of most nanoparticles with sizes surpassing the glomerular filtration threshold. To justify their results, the researchers proposed that, the small diameters of CNTs were excreted in urine despite they were long. Still, this hypothesis is debatable. For example, the protein bio-distribution and excretion behavior of quantum dots (QDs) reported by Choi *et al.* it is discovered that the maximum of ~6 nm size of spherical QDs including coatings were allowed to fast urinal excretion. However, the QDs are much smaller than the diameter of SWCNT bundles (10-40 nm) or MWCNTs (20-80 nm) Lacerada *et al.* (2008) used in these bio-distribution studies. Hence, the reported fast urinal excretion of CNTs obliges validation. Several other labs have also evaluated the bio-distribution of radio labeled CNTs in mice. Wang *et al.*, acknowledged slow urinal excretion and low RES uptake in their first study. However, successive reports by the same group using ^{14}C-taurine functionalized CNTs, showed persistent liver accumulation of CNTs after intravenous injection (Deng *et al.* 2008). A study conducted by McDevitt *et al.* (2007) using antibody conjugated radio labeled CNTs functionalized by 1,3-dipolar cyclo addition also showed slow urinal excretion and high CNT uptake in the liver and spleen McDevitt *et al.* 2007. The bio-distribution studies of radio labeled, PEGylated SWNTs, revealed uptake of SWNT in RES organs without fast clearance (Liu *et al.* 2007). A considerable amount of CNTs in remained in the body even after 15 days. The radiolabel method is a suitable method to detect the bio-distribution of a substance, but may lead to false results, if excess free radioisotopes in the radio labeled CNT samples not removed completely. The free radioisotopes are small molecules that could be fast excreted through urine after intravenous injection. Furthermore, radiolabels could be steadily liberated from CNTs *in vivo*, and be steadily excreted in the free form. Hence, radio labeling is not an ideal strategy to analyze the excretion and long-term fate of CNTs. Scientist have learned that the photoluminescence is the intrinsic properties of CNTs. Cherukuri *et al.* (2006)

used Individual semiconducting SWNTs which exhibit NIR photoluminescence, to track nanotubes in rabbits. Without finding detailed bio-distribution data, the authors could not witnessed SWNT photoluminescence signals in any organs except liver.

Yang *et al.* 2007 carried out the study to see the biodistribution of ^{13}C enriched unfunctionalized SWNTs over a month using isotope ratio mass spectroscopy. The result showed high nanotube uptake in liver, lung, and spleen without noticeable excretion within 28 days. Raman spectroscopy has been used to study the long-term fate of PEGylated nanotubes in mice. It was revealed that most of the PEGylated SWNTs were accumulated in the liver and spleen after intravenous injection, but slowly excreted via the biliary pathway into the feces within months. A weak SWNT Raman signal also detected in the mouse kidney and bladder. It is revealed that small portion of SWNTs with short lengths was excreted through urine.

4. *IN VITRO* TOXICITY OF CARBON NANOTUBES

In vitro toxicological studies are a very important tool for nanotoxicology, compared to *in vivo* studies.Because,lower cost, minimizing ethical concerns and reducing the number of laboratory animals required for testing.

4.1 CNT toxicity studies in animal cell lines

The issue of carbon nanotubes toxicity is still unsettled even in cell culture experiments. Inhibition of HEK 293 cell proliferation after exposure to SWCNTs (Cui *et al.* 2005), MWCNTs induce cell cycle arrest and increase apoptosis/ necrosis of human skin fibroblasts Ding *et al.* (2005) were observed by different research groups. However, it is worth mentioning that, functionalized CNTs were not used in those studies. Bottini *et al.* (2006) observed T lymphocytes apoptosis provoked by oxidized MWCNTs. However, simple oxidation, used in these studies, is not enough to disperse carbon nanotubes in saline and cell culture media, so it is not a kind of biocompatible functionalization. Sayes *et al.* (2006) indicated that the toxicity of CNTs also dependent on the density of functionalization. Least toxicity was observed for those functionalized with the high density of phenyl-SO_3X groups. These results are comprehensible, because CNTs without proper functionalization carry a highly hydrophobic surface. Consequently, they may aggregate in the cell culture medium. The aggregation of CNTs leads to binding of various biological species, including proteins, via hydrophobic interactions, induces cell toxicity. Khalid *et al.* (2014) reported no toxicity of functionalized MWCNT to Saos cell lines up to the tested concentration of 1000 µg/ml. Other factors like surfactants may also play a role in the observed toxicity of CNT *in vitro*. Extra surfactants, present in the CNT suspensions, are known to be highly toxic to cells (Dong *et al.* 2008). The metal catalyst,used during the synthesis of CNTs, should also be consideredas an important factor when the toxicity of carbon nanotubes is studied (Plata *et al.* 2008). Moreover, suitableanalytical methods must be hired in toxicity analysis to preventinterference of carbon nanotubes with the test reagents (Casey *et al.* 2007). Davoren *et al.* (2007), reported concentration dependent cytotoxicity of SWCNT on a lung-carcinoma cell line (A549). Another study, led by Sharma, revealed that SWCNT induced oxidative stress in rat lung cells (Sharma *et al.* 2007). Herzog *et al.* (2007) reported the same oxidative stress linked alterations in primary bronchial epithelial cells and A549 cells but the study also revealed that the reaction is strongly dependent on the dispersion medium used (Herzog *et al.* 2007). Pulskamp, used two cells lines (human A549 and rat macrophages NR8383) and tested with CNTs and revealed as oxidative stress were induced to these cell lines. However, when purified SWCNT were compared with commercial CNT, it is revealed that all the biological effects are associated with the metal traces. There is a confusing result between WST 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt)and MTT (3-(4,5-Dimethylthiazol - 2 - yl) - 2, 5 - diphenyltetrazolium bromide) viability assays. These dies depend on the mitochondrial dehydrogenases activity (Pulskamp *et al.* 2007). The variations can only be described based on associations of CNT with non-soluble formazan crystals in MTT.That is why, a suitable assay methods and well-characterized materials is the most important requirements for *in vitro* toxicity assays of carbon nanotubes.

4.2 CNT toxicity studies in bacteria and yeast cells

As an alternative to animal cells lines, bacteria and yeast can be a valuable model for investigating how single celled microorganisms respond to the

environmental stressors such as CNTs (Boor, 2006). Numerous toxicity mechanisms have been proposed for CNT, comprising, disruption/ penetration of the cell envelope, oxidation of cell components, interruption of trans membrane electron transfer, and formation of secondary products such as reactive oxygen species (ROS) or dissolved heavy metal ions (Kaang *et al.* 2007).Toxicity of a CNT is dependent on its composition along with its geometry and surface functionalization. Numerous studies have indicated that well functionalized, serum-stable CNTs are safe to animal cells, whereas CNTs without functionalization appeared severely toxic to human or animal cells lines at moderate dosage (Dumortier *et al.* 2006).The SWCNT carry a strong antimicrobial action for both suspended and deposited bacteria, and disturb the formation of bacterial films. The direct interaction between the SWCNT and bacteria is probable the main reason to cause cell death (Kang *et al.* 2007). Well-dispersed individual SWCNT are more toxic than agglomerates due to better physical puncturing of bacterial membranes and ruin the cell integrity (Liu *et al.* 2009). The CNT-bacteria interaction is influenced by functionalization and length of CNT. It may regulate the toxic effect also. A negatively charged or neutral SWCNT functionalized with -OH or -COOH aggregates more proficiently with bacteria and decreases bacteria viability as compared to the positively charged SWCNTs functionalized with -NH_2 (Arias *et al.* 2009). Similarly, longer SWCNTsinduced concentration and time dependent toxicity to bacteria.Whereas, short SWCNT were less toxic as they aggregated themselves (Yang *et al.* 2010). Purity of SWCNTs may also affect bacterial toxicity. Pure SWCNTs was found to be less toxic than SWCNTs with higher metal content due to glutathione oxidation occurred shortly after contact (Vecitis *et al.* 2010). Additionally, higher ionic strength suspensions, such as phosphate buffered saline or brain heart infusion broth, also reduces SWCNT toxicity due to reduced intensity of interactions between SWCNT and cells, compared to low ionic strength suspensions (deionized water or saline). Likewise, coating withnatural organic matter (NOM) decreases SWCNT toxicity, despite reduced aggregation (Kang *et al.* 2009). Another studies revealed that, SWCNT decreases enzyme activity and microbial biomass at concentration 300 mg kg^{-1} and higher (Jin *et al.* 2013). Asit is clear that SWCNT induces bacterial death, a surface coating with SWCNT would reduce biofilm formation in both natural and industrial environments (Rodrigues *et al.* 2010). The MWCNT seems to be less toxic to bacteria as compared to SWCNT (Kang *et al.* 2008). The reduced toxicity may be due to lesser interactions between bacteria and MWCNT. The less interaction might be due to the higher rigidity and probably lesser van der Waal's forces at the MWCNT surface. Thin MWCNT with smaller diameter produces higher toxicity to bacteria compare to larger ones (Zheng *et al.* 2010). When the effect of length of MWCNT was assessed, shorter MWCNT were more toxic to *Pseudomonas fluorescens* compared to long MWCNT (Riding *et al.* 2012). When MWCNT are uncapped, debundled and dispersed in solution, the toxicity to bacteria increased (Kang *et al.* 2008). The purity of CNT has also been advocated to affect the toxicity in microorganisms. However, when the toxicity between pristine and purified MWCNT were compared in two bacterial strains (*Escherichia coli* and *Cupriavidus metallidurans*), no difference in toxicity of MWCNT was observed between the two forms (Simon *et al.* 2009). Heating purification of CNs probably has limited capacity to alter the surface compared to acid treatment, thus upholds toxicity of the raw form. However, in both the studies, CNTs were suspended in Gum Arabic (GA, 0.25 wt %), which might have modified the surface, affecting the toxicity. During soil toxicity assay, MWCNT reduced microbial biomass and enzyme activity at concentration 5000 mg kg^{-1} (Chung *et al.* 2011). In a separate study, the conidia of the fungi *Paecilomyces fumosoroseus* were incubated for 865 h with 0.2 mg L^{-1} raw and/or carboxylated MWCNT. Mycelium growth on solid medium was observed after incubation. Contact between the fungi and CNTs had no significant effect on growth and biomass production, but reduction of biomass was observed after exposure to raw MWCNT for 865 hrs (Gorczyca *et al.* 2009). Mechanical effects of CNT, as witnessed in bacteria, might have induced the effects.

5. ECOTOXICITY OF CARBON NANOTUBES

As the production and extensive application of CNTs in industrial and customer products is increasing, the release of this nanomaterial into the environment also will rise. Various scientific reviews have assessed the sources, behavior, fate, and the mechanisms of toxicity of carbon nanomaterial. Most of these assessments realized that further research is compulsory in the field of nano-ecotoxicology Table 1**.**

Table 1. Summary of the studies related to eco-toxicity of the CNTs on different organisms

Organism tested	Types of CNTs	LOEC	EC 50	Mechanism of toxicity	References
Chlorella vulgaris	Pristine CNT	0.053 mg L^{-1}	1.8 mg L^{-1}	Oxidative stress, agglomeration and physical interactions	(Schwab *et al.* 2011)
	MWCNT of diameter 10, 20–40, and 60–100 nm	NA	41.0,12.7, and 12.4 mg L^{-1}, respectively	Oxidative stress, agglomeration and physical interactions	(Long *et al.* 2012)
Pseudokirchneriella subcapitata	Pristine CNT	0.053 mg L^{-1}	2.5 mg L^{-1}	Oxidative stress, agglomeration and physical interactions	(Schwab *et al.* 2011)
	SWCNT	0.25 mg L^{-1}	NA	Oxidative stress, agglomeration and physical interactions	(Youn *et al.* 2011)
Thalassiosira pseudonanas	DWCNTs	0.1 mg L^{-1}	1.86 mg L^{-1}	Oxidative stress, agglomeration and physical interactions	(Kwok *et al.* 2010)
Dunaliella tertiolecta	MWCNT	NA	0.8 mg L^{-1}	Oxidative stress and photosynthesis inhibition	(Wei *et al.* 2010)
Tetrahymena thermophila	SWCNT	1.6 mg L^{-1}	NA	Physical interactions	(Ghafari *et al.* 2008)
Stylonychia mytilus	Functionalized MWCNT	1 mg L^{-1}	NA	Physical interactions	(Zhu *et al.* 2006)
Hydra attenuata	SWCNT	1–10 mg L^{-1}	NA	Physical interactions	(Calabrese *et al.* 2005)
Daphnia magna	SWCNT (60% pure)	NA	1.3 mg L^{-1}	Physical interactions	(Zhu *et al.* 2009)
	MWCNT resuspended in NOM	NOEC 20 mg L^{-1}	NA	No toxicity	(Kim *et al.* 2009)
	MWCNT grafted with polyethylenimine	NA	25 mg L^{-1}	Increased size of the surface coating	(Petersen *et al.* 2011)
Ceriodaphnia . dubia	MWCNT resuspended in NOM	0.25 mg L^{-1}	NA	Agglomeration	(Li *et al.* 2011)
Danio rerio embryo	SWCNT	120 mg L^{-1}	NA	Agglomeration	(Cheng *et al.* 2007)
Oryzias melastigma	DWCNT	10 mg L^{-1}	NA	Agglomeration	(Kwok *et al.* 2010)
Xenopus leavis larvae	DWCNT	10 mg L^{-1}	NA	Physical interactions	(Bourdiol *et al.* 2013)
Drosophila melanogaster	SWCNT and MWCNT in 1 g kg^{-1} food	--------	----------	No toxicity	(Leeuw *et al.* 2007)
Female Fisher rats	Oral gavage of 0.64 mg kg^{-1} SWCNT	NA	NA	Increased levels of oxidative damage to DNA in liver and lung tissue	(Aschberger *et al.* 2010)
Sprague–Dawleyrat	1000mg kg^{-1} of SWCNT from gestation day 6 to 19	NA	NA	No teratogenicity	(Lim *et al.* 2011)

Abbreviations: LOEC: Least observable effect concentration, EC 50: Effective concentration 50, NOEC: No observed effect concentration, NOM: Natural organic matter.

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